Bot. Bull. Academia Sinica **13**: 92-103 (1972)

NATURE OF DRUG TOLERANCE IN *SCLEROTIUM ROLFSII*[1]

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Abstract

Tolerant sectors of *Sclerotium rolfsii* appeared about 21 days after prolonged exposure to sublethal levels of pentachloronitrobenzene (PCNB). However, loss of tolerance was also observed in most of tolerant isolates except that S-1T and S-7T retained their ability of tolerance through several transfers on Joham's agar free of PCNB. Both isolates exerted higher pathogenic and saprophytic activities than parent isolates from which they originated. This fact and changes in aversion phenomena indicate that they are true mutants.

Higher yield of oxalic acid by tolerant isolates might play a role in the pathogenicity. The possibility that the cause of tolerance might involve detoxification by the fungus was not ruled out since the loss of PCNB in cultures of tolerant isolates was greater than that in cultures of sensitive isolates.

Introduction

In the pervious study, it was found that there was tolerance developed in *Sclerotium rolfsii* induced by an organic fungicide, PCNB (Pentachloronitrobenzene) (Yang and Wu, 1971), which is known as the most effective and widely used chemical for control of the diseases caused by this particular fungus of economic importance (Aycock, 1966).

Tolerance of fungi to organic fungicides is by no means unusual (Corden, 1969; Georgopoulos and Zaracovitis, 1967). In 1940, Farkas and Aman found the diphenyl tolerance of *Penicillium digitatum* (Georgopoulos and Zaracovitis, 1967). Seven years later, Roy reported the development of tolerant strain from *Botrytis cinerea* exposed to chlorinated nitrobenzene (Priest and Wood,

(1) This work was supported by the Agricultural Research Center and by the National Science Council, Rep. of China.

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1961). Since then a number of workers have obtained tolerant strains of certain susceptible fungi after prolonged cultures on sublethal levels of the fungicides. Sinclair (1960) was the first to report that naturally occurring isolates of *Rhizoctonia solani* from cotton seedlings varied in their ability to grow on agar containing different concentrations of Pentachloronitrobenzene (PCNB). In the same year, Georgopoulos and Thanasoulopoulos (1960) found a PCNB-tolerant strain produced by *Sclerotium rolfsii* grown on potato-dextrose agar containing 0.02% Terraclor 75 W. P. Eckert (1962) evaluated fungistatic and phytotoxic properties of 19 derivatives of nitrobenzene and suggested that the chlorinated nitrobenzenes were "structurally nonspecific" toxicant and the impaired permeability might be a cause of tolerance. However, Georgopoulos (1964) found that the mycelial growth of tolerant strains of *S. rolfsii* was enhanced by the addition of chlorinated nitrobenzenes.

Despite of the fact that tolerant strains are readily obtained in exposure to the chlorinated nitrobenzenes, relatively little is known concerning the mode of toxic action of these compounds (Corden, 1969; Dekker, 1969; Georgopoulos and Zaracovitis, 1967). Furthermore, *S. rolfsii*, after exposure to sublethal levels of PCNB, tended to increase their survivability in and virulence to peanut plants (Yang and Wu, 1971). The increased ability for survival and virulence of the fungus is worthy of further study since the control mechanism of the tolerance, survivability and virulence of this particular fungus induced by PCNB is a subtle problem for the fungicide to perform well in the field. We report our study on these aspects.

Materials and Methods

The isolates S–1 and S–7 of *Sclerotium rolfsii* Sacc. were originally isolated from diseased soybean which were collected from the Kaohsiung District Agricultural Experiment Station (Yang and Wu, 1971). The tolerant mutants, S–1T and S–7T were derived respectively from isolates S–1 and S–7 growing under sublethal dosage of PCNB. Joham's medium (0.0015 M $MgSO_4$, 0.004 M K_2HPO_4, 0.002 M KCl, 0.0125 M NH_4NO_3, 0.1 ppm thiamine chloride, 2.0 ppm $FeSO_3$, 2.0 ppm $MnSO_4$, 2.0 ppm $ZnSO_4$, and 4.0% Glucose) was used throughout the experiments. For the solid medium, 20 grams agar were added to a liter of Joham's liquid medium. Brassicol (PCNB) 75 Wettable Powder of Hoechst AG, sodium Pentachlorophenate (PCP) and *p*-dichlorobenzene (DCB) of Wako Pure Chemical Industries, Ltd., glutathione (Reduced) of National Biochemical Corporation, and L-cysteine of Sigma Chemical Co. were used. The stock solutions of all the chemicals were made by dissolving the appropriate amounts of chemicals in sterile water, except that DCB was dissolved in 95% ethyl alcohol.

The fungus was grown on Joham's medium with or without agar, in which PCNB were incorporated in amounts required for the experiments. The inoculum was usually made by cutting the margin of 3 day old colony grown on Joham's agar, unless otherwise mentioned. All the cultures were incubated at 28°–31°C for a given period of time under fluorescent light (20 W×2) with a distance about 30 cm from light source. Radial growth of the fungus was measured at 24 hours intervals. Dry weight of the mycelium was measured by drying fungus felts at 120°C overnight to obtained a constant weight.

Stationary cultures grown in 125 ml flask containing 20 ml of Joham's liquid medium were used to analyze the production of oxalic acid and loss of PCNB during the incubation under the experimental condition mentioned above. In order to obtain uniform inoculum, 40 ml Joham's liquid medium were added to a given amount of mycelial felt in a stainless steel jar and ground for 15 seconds in a Waring Blendor. Two ml of inoculum, macerated mycelium, were pipetted to a flask containing 16 ml Joham's liquid medium and 2 ml PCNB stock solution to make up the total volume of 20 ml for each flask. They were carried aseptically.

The production of oxalic acid was determined according to the method described by Bateman and Beer (1965). Mycelial felts were removed by centrifugation at 1,500 g for 10 minutes. The supernatant was poured back to a 125 ml flask containing 10 ml calcium chloride-acetate buffer, 10% calcium chloride dissolved in 12.5% acetic acid, mixed, and allowed to stand overnight. The sediment was obtained by centrifugation at 3,000 g for 10 minutes and washed with 10 ml of 5% acetic acid solution saturated with calcium oxalate and again centrifuged. The supernatant was discarded and 8 ml 4 N H_2SO_4 were added to dissolve the sediment. The mixture was poured to a 125 ml flask on a hot plate with a magnetic stirrer, heated to 90°C, and titrated with 0.02 N potassium permanganate to a faint pink-colored end point. The amount of oxalic acid was calculated as follows: 1.02456× volume of 0.02 N $KMnO_4$ used in ml=mg oxalic acid.

The amount of PCNB was determined by gas chromatography. Preliminary results indicated that the following procedure could recover 93% of the added PCNB from Joham's liquid medium. Stationary cultures of *S. rolfsii* incubated at 28°–31°C for 4 days and 21 days were homogenized with a Sorvall omni-mixer for 2 minutes. The homogenates were extracted twice with equal volume of benzene. Benzene was removed by a flash evaporator at 40°C. The residue was dissolved in 5 ml of benzene and stored in a refrigerator at 5°C before analysis. A Varian Aerograph model-1800 gas chromatograph with flame ionization detector was used to analyze PCNB. A column containing 5% DC-200 on Chromosorb W was used. Injection and detection temperatures

were 225°C and the flow rate of nitrogen was maintained at 30 ml/min. The column temperature was 195°C.

The Warburg apparatus was used to determine oxygen uptake, following the standard procedures (Umbreit *et al.*, 1957). Equilibration time was 10 minutes and the temperature was at 30°C. Each flask contained 2.0 ml total volume of reaction mixture, including 1.8 ml Joham's liquid medium and 0.2 ml PCNS at a given concentration. The PCNB suspension was added to the flask side arm. For the control, distilled water was used instead of PCNB suspension. In order to observe the effect of PCNB vapour, all the flasks were run for one hour before tipping PCNB into the Joham's liquid medium. Thereafter continuous reading was made at 10 minutes intervals for 2 hours before experiments were terminated. Four days old cultures of *S. rolfsii* grown on Joham's agar plate were used for the measurement of oxygen uptake. Each flask contains 3 pieces of mycelial felts from which attached agar has been removed.

The pathogenicity test was carried out as described in the previous report (Yang and Wu, 1971). Peanut seeds were treated with 0.1% $HgCl_2$ for 2 minutes and washed in 5 changes of sterile water. The treated seeds were placed on water agar (7.5 grams agar per liter of distilled water) in a test tube and allowed to grow for 7 days under fluorescent light (20 W×2) at 28°–31°C. Finally, 2 ml macerated mycelium were pipetted to the agar surface near to the peanut stem and the disease index was recorded 7 days after inoculation.

Aversion phenomenon and saprophytic activity in soil were observed by the methods described by Liu and Wu (1971). Joham's agar plates were used to determine the aversion phenomenon. To each plate was seeded two pieces of mycelial disk (3 mm in diameter) of different or same isolates 4 cm apart. The aversion phenomenon was defined as the formation of barrage by which two colonies were separated without overlapping growth of mycelia. For the determination of saprophytic activity, 20 grains of sorghum seeds were poured into a 125 ml flask with 50 g sandy loam and autoclaved at 130°C for 30 minutes. After cooling to room temperature, sclerotia of isolates S–1 and S–1T or S–7 and S–7T were transferred to the sterilized soil, 10 sclerotia for each, then 10 ml sterile water were added and incubated at 28°–31°C for 6 days. The sorghum seeds were washed with running water to remove soil and placed on Joham's agar plate inoculated either isolate S–1 or S–7 toidentify the number of sorghum seed affected by observing the aversion phenomenon.

Results

Induction and growth of tolerant mutants

In order to obtain tolerant sectors developed from the edge of the abnormal

Table 3. *Response of PCNB sensitive and tolerant isolates to sodium Pentachlorophenate (PCP) and p-dichlorobenzene (DCB) at different concentrations incubated at 28°–31°C for 4 days*

Chemical	Concentration (ppm)	Diameter (mm)* of colony			
		S-1	S-1T	S-7	S-7T
PCP	0	85.3	22.3	90.0	40.0
	10	3.0	38.6	72.6	25.3
	100	3.0	22.0	35.0	3.0
	1,000	3.0	3.0	3.0	3.0
	2,000	3.0	3.0	3.0	3.0
DCB	0	3.0	6.6	3.0	3.0
	10	3.0	4.0	4.0	10.6
	100	3.0	3.0	3.0	3.0
	1,000	3.0	5.3	3.0	4.6
	2,000	3.0	3.0	3.0	3.0

* Data are the averages of triplicates.

Table 4. *Effect of reduced glutathione (GSH) and cysteine on the radial growth of PCNB sensitive and tolerant isolates on Joham's agar containing 250 ppm PCNB incubated at 28°–31°C for 4 days*

Isolate	Sensitivity to PCNB	Diameter (mm)* of colony at concentrations (ppm)					
		GSH			Cysteine		
		0	100	400	0	100	400
S-1	Sensitive	8.0	9.3	9.0	10.0	9.6	8.6
S-1T	Tolerant	34.6	36.6	38.6	48.0	39.3	36.0
S-7	Sensitive	10.3	9.0	9.3	11.0	9.3	11.0
S-7T	Tolerant	33.0	35.0	41.3	31.0	38.6	41.3

* Data are the averages of six replicates.

Loss of PCNB in cultures

As the tolerant sectors appear 21 days after exposing to lethal levels of PCNB, it is plausible to assume that there is a loss of PCNB during the course of incubation. Since S-1T and S-7T were respectively isolated from the tolerant sectors appeared on the agar plates containing 250 and 500 ppm PCNB, different amounts of PCNB were used for these two isolates. The experimental results indicated that the amount of PCNB in the flask growing tolerant isolates were lower than those growing sensitive isolates though the control flasks also lost their PCNB contents during the course of incubation (Table 5).

colonies, two PCNB sensitive isolates of *Sclerotium rolfsii*, S-1 and S-7, were grown on Joham's agar plates containing 25, 50, 100, 250, 500 ppm PCNB. Tolerant sectors appeared about 21 days after seeding mycelial disk. As shown in Table 1, production of tolerant sectors were frequently found among the colonies grown on the plates impregnated 250 ppm PCNB. Most of the tolerant isolates lost their resistance to PCNB after transfer to fungicide-free medium, whereas only two isolates, S-1T and S-7T, retained their drug tolerance so far.

Table 1. *Induction of Tolerant Mutants of* ***Sclerotium rolfsii*** *on agar plates containing different concentrations of PCNB**

Isolate	Number of sectors appearing at concentrations (ppm)		
	100	250	500
S-1	5	11	4
S-7	5	13	4

* Tolerant sectors were recorded 21 days after seeding of mycelial disk (3 mm in diameter) on Joham's agar at 28°-31°C.

Tolerant isolates, S-1T and S-7T, were characterized by a growth-rate faster than those of sensitive isolates, S-1 and S-7, from which they originated when they were grown on the agar plates impregnated with PCNB (Table 2). However, they grew slower than those of sensitive parent isolates on PCNB-free plates.

Table 2. *Radial growth of PCNB sensitive and tolerant isolates on Joham's agar containing different concentrations of PCNB incubated at 28°-31°C for 4 days*

Isolate	Sensitivity to PCNB	Diameter (mm)* of colony at concentrations (ppm)				
		0	10	100	1,000	2,000
S-1	Sensitive	85.3	20.0	11.0	5.6	3.0
S-1T	Tolerant	22.3	66.0	49.0	26.3	25.6
S-7	Sensitive	90.0	14.0	12.0	7.0	3.0
S-7T	Tolerant	40.0	49.6	47.6	25.6	31.3

* Data are the averages of triplicates.

As shown in Table 3, one of the tolerant isolates, i. e., S-1T, seems to exert the resistance to sodium pentachlorophenate (PCP), whereas the rest of tested isolates show sensitivity to the PCP produced by the hydrolysis of PCNB (Burchfield and Storrs, 1969). On the other hand, both tolerant isolates, S-1T and S-7T, revealed inconsistant response to either reduced glutathione or cysteine in the agar plates impregnated with 250 ppm PCNB (Table 4).

Table 5. *Loss of PCNB in Joham's liquid medium incubated at 28°–31°C*

Isolate	Sensitivity to PCNB	Amount (mg/flask)* of PCNB recovered after		
		0-day	4-day	21-day
S-1	Sensitive	5.5**	3.851 (70.02)***	0.930 (16.91)***
S-1T	Tolerant	5.5	4.097 (74.49)	0.628 (11.42)
Control		5.5	3.500 (63.64)	1.075 (19.55)
S-7	Sensitive	11.0	8.262 (75.11)	1.945 (17.68)
S-7T	Tolerant	11.0	6.702 (60.92)	1.275 (11.59)
Control		11.0	5.786 (52.60)	2.509 (22.81)

* Data are the averages of six replicates.

** Recovery of PCNB added to Joham's liquid medium was about 93%, therefore all the data were culculated by this factor.

*** Figures in parentheses indicated the percentage of recovery based on the amount at 0-day.

Oxalic acid production

Tolerant isolates exerted higher production of oxalic acid on stationary cultures in the lapse of incubation time. However, the difference in yield of oxalic acid between sensitive and tolerant isolates is not consistant, though each experiment consists of six replicates (Table 6). On the other hand, the yield of oxalic acid of the tolerant isolates is markedly greater than that of the sensitive isolates when the cultures are grown in the presence of PCNB (Table 7).

Table 6. *Oxalic acid production of PCNB sensitive and tolerant isolates grown on Joham's liquid medium incubated at 28°–31°C*

Isolate	Sensitivity to PCNB	Oxalic acid production (mg/flask)*				
		0-day	1-day	2-day	3-day	4-day
S-1	Sensitive	1.110	5.165	8.479	9.122	13.358
S-1T	Tolerant	0.909	7.015	8.177	13.375	15.965
Difference		0.201	1.850	0.302	4.253	2.607**
S-7	Sensitive	0.724	6.713	14.754	13.930	8.511
S-7T	Tolerant	0.623	8.277	15.394	27.002	39.384
Difference		0.101	1.564	0.640	13.072	30.873***

* Data are the averages of six replicates.

** LSD=3.549 (0.05), 5.048 (0.01).

*** LSD=8.220 (0.05), 11.692 (0.01).

Table 7. *Effect of PCNB on the oxalic acid production of PCNB sensitive and tolerant isolates incubated at 28°-31°C for 6 days*

Isolate	Sensitivity to PCNB	Oxalic acid production (mg/flask)* at concentrations (ppm)		
		100	250	500
S-1	Sensitive	22.020	6.175	10.531
S-1T	Tolerant	43.743	15.152	18.523
Difference		21.723**	9.077***	7.992****
S-7	Sensitive	10.682	9.269	10.268
S-7T	Tolerant	32.801	21.548	19.278
Difference		22.119+	12.279++	9.010+++

* Data are the averages of six replicates.
** LSD=3.467 (0.05), 4.931 (0.01).
*** LSD=1.496 (0.05), 2.127 (0.01).
**** LSD=3.090 (0.05), 4.394 (0.01).
\+ LSD=8.729 (0.05), 12.416 (0.01).
++ LSD=2.040 (0.05), 2.091 (0.01).
+++ LSD=3.285 (0.05), 4.673 (0.01).

Respiratory changes

Effect of PCNB on the respiration of sensitive isolates were greater than that of tolerant isolates (Table 8). Tipping of PCNB suspension from side arm of a flask did not change the respiration rate seemed to imply that effect of PCNB vapour might be exist under the present experimental condition. Both endogenous respiration and exogenous respiration with glucose in phosphate buffer failed to exert the effect of PCNB on either sensitive or tolerant isolates.

Table 8. *Effect of PCNB on the respiration of PCNB sensitive and tolerant isolates in Joham's liquid medium at 30°C*

Concentration of PCNB (ppm)	Oxygen uptake (μl/mg dry wt. of mycelium/hr.)*			
	S-1	S-1T	S-7	S-7T
0	2.36	1.36	2.55	2.28
100	2.17	—	3.04	—
250	2.03	—	5.54	—
500	4.07	1.65	2.68	2.65
1,000	2.85	1.63	2.35	2.31
2,000	—	1.84	—	2.31
3,000	—	1.48	—	1.72

* Data are the averages of six replicates; —, not determined.

Changes in the aversion phenomenon and saprophytic activity

There was aversion between sensitive isolates S-1 and S-7. However, both tolerant isolates exerted aversion against their parent isolates regardless of their origin although there was no aversion between the tolerant isolates (Table 9). Their ability of aversion was not changed after passage of the host plants (peanut seedling). Pathogenicity test showed that the tolerant isolates retained their pathogenicity which was greatly higher than that of the sensitive isolates from which they originated. Nevertheless, the former also exerted higher saprophytic activity than the latter in the soil.

Table 9. *Changes in the aversion phenomena of PCNB tolerant mutants of Sclerotium rolfsii*

Isolate	S-1	S-1T	S-7	S-7T
S-1	−*	+	+	+
S-1T	+	−	+	−
S-7	+	+	−	+
S-7T	+	−	+	−

* −, no aversion; +, aversion occurred.

Discussion

In the present investigation, two approaches are in use to study the nature of tolerance development. The first consists of induction of tolerant mutation by growing *Sclerotium rolfsii* in the presence of PCNB, modification of PCNB by enzymic and non-enzymic means, and assessment of virulence by plant inoculation and production of oxalic acid. The second approach involves the studies on genetic development of tolerance by determination of aversion phenomenon in relation to saprophytic activity since the perfect stage of this fungus is rarely found on artificial media.

Georgopoulos and Thanasoulopoulos (1960) was the first to report the existance of PCNB tolerance in *S. rolfsii.* They found that the tolerant strain appeared after exposure of the parent isolate to PCNB for only 5 days. In our laboratory, we found that the tolerant sectors appeared about 21 days after prolonged exposure to sublethal levels of PCNB. However, loss of tolerance was also observed in the most of the tolerant isolates except that S-1T and S-7T retained their ability of tolerance to PCNB through several transfers on Joham's agar without PCNB. They grew well on the agar medium containing 3,000 ppm PCNB without alternation of culture characters regardless of the concentrations at which they were obtained. The appearacce of the colonies was similar to that of the parent isolates whereas the sclerotial

formation was impaired when the concentration of PCNB was above 750 ppm. Elsaid and Sinclair (1964) found that adapted tolerance of PCNB by *Rhizoctonia solani* was temporary. Loss of tolerance was also observed only in part of the thallus of *S. rolfsii* by Georgopoulos (1964).

In comparison with the results obtained, it is appearent that tolerant isolates, S-1T and S-7T, do not completely agree with the tolerant strain obtained by Georgopoulos (1960). In fact, both isolates exerted stronger pathogenic strength and saprophytic activity than those of parent isolates from which they originated. Furthermore, changes in aversion phenomena indicate that they are true mutant though the genetic nature of the aversion phenomenon is not clear.

Georgopoulos (1964) showed that the tolerant strain of *S. rolfsii* was also tolerance to the other chlorinated nitrobenzenes. However, our tolerant isolates were not tolerant to sodium pentachlorophenate (PCP) and *p*-dichlorobenzene (DCB) though a chloroneb-resistant mutant of *Ustilago maydis* was resistant to PCNB, PCP, DCB, and others. Burchfield and Storrs (1958) studied the apparent reactivities of metabolites and related compounds containing sulfhydryl groups with aromatic fungicides and found that glutathione reacted as well as cysteine. Both compounds apparently shifted the absorption maximum in the range of 288 to 370 nm. Since reduced glutathione was involved in the detoxication of dichloronitrobenzene (Booth *et al.*, 1961), inert effect of reduced glutathione (GSH) and cysteine is puzzling. The optimum pH for the enzyme catalysing the reaction in which the chlorine atom was replaced by GSH appeared to be pH 8 which was never found in the cultures under the present experimental condition (Yang and Wu, 1971).

Metabolism of PCNB by rabbit (Betts *et al.*, 1955) and microorganisms (Chacko and Lockwood, 1966; Ko and Farley, 1969; Nakanishi and Oku, 1969) have been extensively studied. The possibility that cause of tolerance might involve the detoxification of PCNB by the *S. rolfsii* was not ruled out since the loss of PCNB in cultures of tolerant isolates were greater than that in cultures of sensitive isolates. However, the control flasks also lost their PCNB in the lapse of time. It might be due to the photo-sensitivity of the compound since all the experiments were carried in the light.

Higher yield of oxalic acid by tolerant isolates might play a role in the virulence since synergistic action between polygalacturonase and oxalic acid was considered to be a significant factor in the rapid destruction of plant tissue by *S. rolfsii* (Bateman and Beer, 1965). In the course of study on oxalate accumulation, glyoxylate dehydragenase activity, and growth of *S. rolfsii*, it was found that the total enzyme activity was sufficient to account for the production of oxalic acid (Maxwell and Bateman, 1968). Since PCNB

was known to inhibit the succinic dehydrogenase in Kreb's cycle (Nakanishi and Oku, 1969), the yield of oxalic acid of the tolerant isolates must be greater in the presence of PCNB.

McCallan *et al.* (1954) compared the effect of fungicides on the oxygen uptake of spores concluded that oxygen uptake-time curves in the presence of fungicides were of three types: a depression increasing with concentration, a depression followed by increase in uptake, and appreciable effect with concentration. Effect of PCNB on the respiration of sensitive isolates was similar to the second type described by McCallan *et al.* (1954). However, the tolerant isolates revealed third type which resembled the results obtained by Torgeson (1963).

Acknowledgment

The authors are indebted to Mr. T. T. Lo, Senior specialist of JCRR, for his encouragement and also to Mr. Chi-young Wang for his technical assistance in gas chromatography.

Literature Cited

AYCOCK, R. 1966. Stem rot and other diseases caused by *Sclerotium rolfsii* or the status of Rolf's fungus after 70 years. N. Carolina Agr. Exp. Sta. Tech. Bull., **174**: 1-202.

BATEMAN, D. F. and S. V. BEER. 1965. Simultaneous production and synergistic action of oxalic acid and polygalacturonase during pathogenesis by *S. rolfsii*. Phytopathology **55**: 204-211.

BETTS, J. J., S. P. JAMES and W. V. THORPE. 1955. The metabolism of pentachloronitrobenzene and 2:3:4:6-tetrachloronitrobenzene and the formation of mercapturic acids in the rabbit. Biochem. J., **61**: 611-617.

BOOTH, J., E. BOYLAND and P. SIMS. 1961. An enzyme from rat liver catalysing conjuations with glutathione. Biochem. J. **79**: 516-524.

BURCHFIELD, H. P. and E. E. STORRS. 1958. Influence of pH and group interactions on the reactivities of metabolites and related compounds containing sulfhydryl groups. Cantrib. Boyce Thompson Inst. **19**: 417-436.

BURCHFIELD, H. P. and E. E. STORRS. 1969. Electronic and steric aspects of fungicidal action. p. 61-100 in D. C. Torgeson (Ed.) Fungicides Vol. 2 Chemistry and Physiology. Academic Press, New York.

CHACKO, C. I., J. L. LOCKWOOD, and M. ZABIK. 1966. Chlorinated hydrocarbon pesticides degradation by microbes. Science **154**: 893-895.

CORDEN, M. E. 1969. Aromatic compounds. p. 477-529 in D. C. Torgeson (Ed.) Fungicides Vol. 2 Chemistry and Physiology, Academic Press, New York.

DEKKER, J. 1969. Acquired resistance to fungicides. World Rev. Pest Control, **8**: 79-85.

ECKERT, J. W. 1962. Fungistatic and phytotoxic properties of some derivatives of nitrobenzene. Phytopathology **52**: 642-649.

ELSAID, H. M. and J. B. SINCLAIR. 1964. Adapted tolerance to organic fungicides by isolates of *Rhizoctonia solani* from seedling cotton. Phytopathology **54**: 518-522.

GEORGOPOULOS, S. G. 1964. Chlorinated-nitrobenzene—tolerance in *Sclerotium rolfsii*. Ann. Inst. Phytopathol. Benaki. N. S. **6**: 156-159.

GEORGOPOULOS, S. G. and C. C. THANASOULOPOULOS. 1960. Research on the control of *Sclerotium rolfsii* Sacc. with fungicides. Ann. Inst. Phytopath. Benaki, N. S. **3**: 65-78.

GEORGOPOULOS, S. G. and C. ZARACOVITIS. 1967. Tolerance of fungi to organic fungicides. Annu. Rev. Phytopathol. **5**: 109-130.

KO, W. H. and J. D. FARLEY. 1969. Conversion of pentachloronitrobenzene to pentachloroaniline in soil and the effect of these compounds on soil microorganisms. Phytopathology **59**: 64-67.

LIU, T. M. and L. C. WU. 1971. Aversion phenomenon in relation to saprophytic activity of *Sclerotium rolfsii* in soil. Sci. Agri. **19**: 393-396.

MAXWELL, D. P. and D. F. BATEMAN. 1968. Oxalic acid biosynthesis by *Sclerotium rolfsii*. Phytopathology **58**: 1635-1642.

MCCALLAN, S. E. A., L. P. MILLER, and R.M. WEED. 1954. Comparative effect of fungicides on oxygen uptake and germination of spores. Cantrib. Boyce Thompson Inst. **18**: 39-68.

NAKANISHI, T. and H. OKU. 1969. Metabolism and accumulation of pentachloronitrobenzene by phytopathogenic fungi in relation to scective toxicity. Phytopathology **59**: 1761-1762.

NAKANISHI, T. and H. OKU. 1969. Mechanism of selective toxicity of fungicide: metabolism of pentachloronitrobenzene by phytopathogenic fungi. Ann. Phytopath. Scc. Japan **35**: 339-346.

SINCLAIR, J. B. 1960. Reaction of *Rhizoctonia solani* isolates to certain chemicals. Plant Dis. Reptr. **44**: 474-477.

TILLMAN, R. W. and H. D. SISLER. 1971. A chloroneb-resistant mutant of *Ustilago maydis*. Phytopathology **61**: 914 (Abstr.).

TORGESON, D. C. 1963. Effect of fungicides on the respiration of three species of soil fungi. Phytopathology **53**: 891 (Abstr.).

UNBREIT, W. W., R. H. BURRIS, and J. F. STAUFFER. 1957. Manometric technique. Ed. 3 Burgess Publ. Co., Mineapolis, 338p.

YANG, W. G. and L. C. WU. 1971. Tolerance to PCNB and simulated pathogenic strength of *Sclerotium rolfsii* Sacc. Mem. Agri. Coll., Natl. Taiwan Univ. **12**: 191-202.

白絹病菌耐藥性的研究

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耐藥變異株在接觸 PCNB 21日發現，但多數菌株再移到不含 PCNB 培養基後，其耐藥性消失，其中僅有兩個菌株經過數次移植後，仍然不失去耐藥性。這些耐藥菌株的病原性和腐生活力都比親代菌株强，並且他們的嫌觸作用改變，所以本試驗所得變異株並非適應藥劑所致，而是 PCNB 誘導變異的結果。變異菌株病原性的增加與草酸的產量具有密切的關係。一般來說，耐藥變異菌株比親代菌株多，尤其是培養液含有 PCNB 時爲甚。耐藥菌株可能分解 PCNB。